

CLAIMS

That which is claimed is:

- 5 1. A method for testing a sample for the presence of a cyanide compound, the method comprising:
 - a) reacting at least one boronic acid containing fluorophore with the sample,
 - b) illuminating the sample and boronic acid containing fluorophore to generate a fluorescent property; and
- 10 10 c) observing the sample with means for detecting the fluorescent property, wherein a change in the fluorescent property indicates the presence of cyanide.
- 15 2. The method according to claim 1 wherein the presence of the cyanide compound at least partially quenches the fluorescent property of the boronic acid containing fluorophore in a dose dependent manner.
3. The method according to claim 1, wherein the presence of cyanide increases the fluorescent property of the boronic acid containing fluorophore.
- 20 4. The method according to claim 1, wherein changes in the fluorescent property is measured by determining the change in intensity or lifetime of fluorescence emission.
5. The method according to claim 4, wherein the degree of change in the fluorescence emission relates to concentration of the cyanide compound and the binding affinity of the cyanide compound to the boronic acid containing fluorophore.
- 25 6. The method according to claim 1, further comprising the step of determining the concentration of the cyanide compound by comparing the fluorescent property with the fluorescent property obtained with known concentrations of the cyanide compound.
- 30 7. The method according to claim 4, wherein the means for observing a fluorescence property response is a camera, a spectrophotometer, a fluorescence microscope, a laser scanner, or a flow cytometer.
- 35 8. The method according to claim 1, wherein the sample comprises living cells or

biological fluids.

9. The method according to claim 1, wherein the sample is a soil or water sample, or is obtained from a soil or water sample.

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10. The method according to claim 1, wherein illuminating the sample comprises using an LED light source having an excitation range of about 320 nm to about to 400 nm.

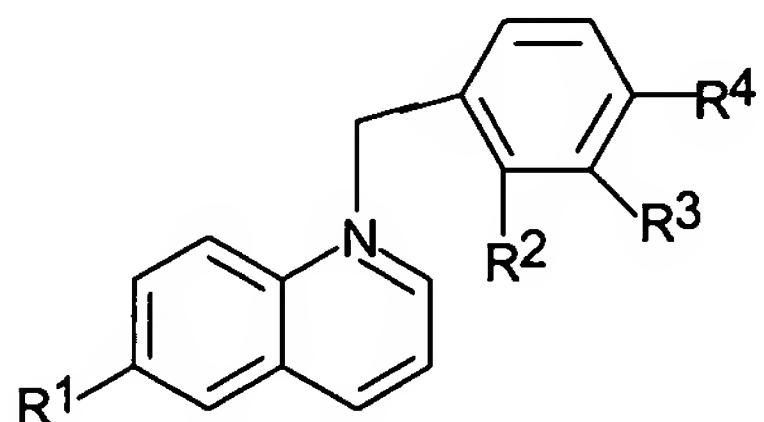
11. The method according to claim 1, wherein the change in fluorescent property is a change 10 in fluorescence emission intensity, fluorescence lifetime, excitation wavelength or emission wavelength.

12. The method according to claim 1, wherein the boronic acid containing fluorophore comprises a heterocyclic quaternary nitrogen (a ring nitrogen) linked through a phenyl ring with 15 a boronic acid moiety.

13. The method according to claim 1, wherein the boronic acid containing fluorophore is

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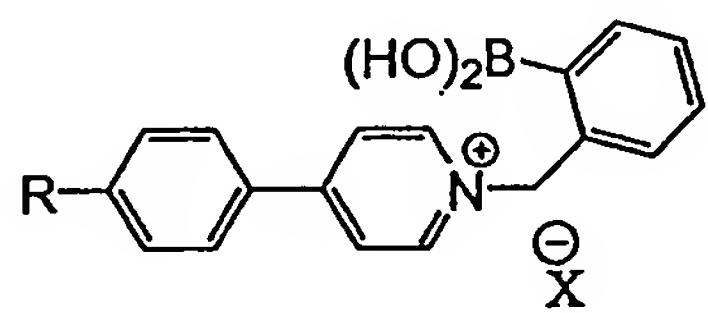
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25 wherein R¹ is H, a straight chain or branched C₁-C₄ alkyl group, C₁-C₄ alkoxy group, aryl group, or an amine group NR⁵R⁶, wherein R⁵ and R⁶ may be the same as or different from one another and is independently selected from the group consisting of H and C₁ - C₄ alkyl groups, and, R², R³ and R⁴ may be the same or different and may be hydrogen or B(OH)₂ with the proviso that the compound comprises one B(OH)₂ group;

(II)

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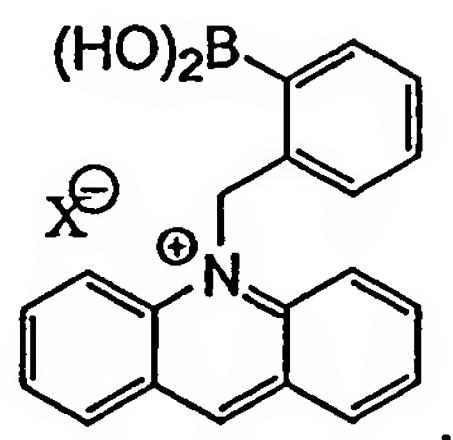


wherein X is chloride, bromide or iodide, and R is selected from the group consisting of H, straight chain or branched C₁-C₄ alkyl group, C₁-C₄ alkoxy group, aryl group, hydroxyl, sulfonyl,

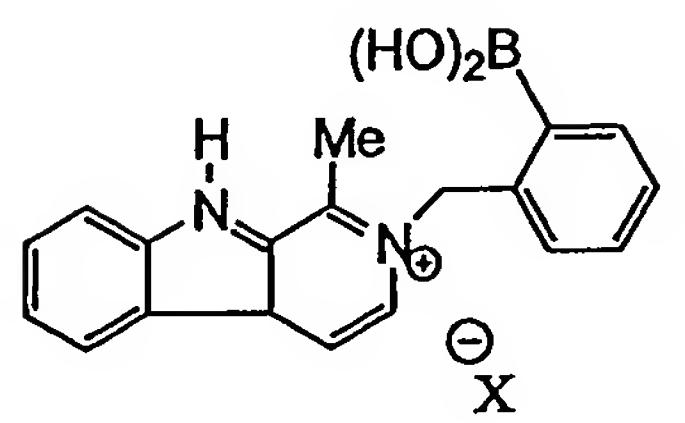
and NR^5R^6 , wherein R^5 and R^6 may be the same as or different from one another and is independently selected from the group consisting of H and $C_1 - C_4$ alkyl groups;

(III)

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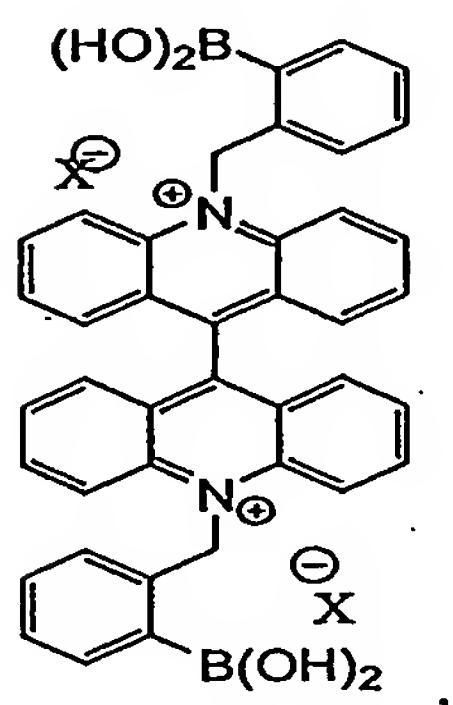


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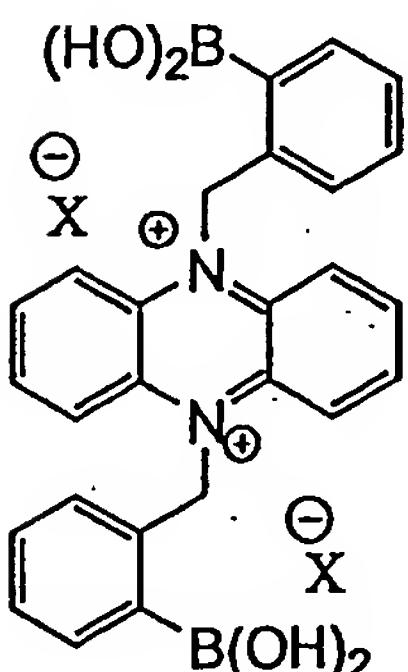


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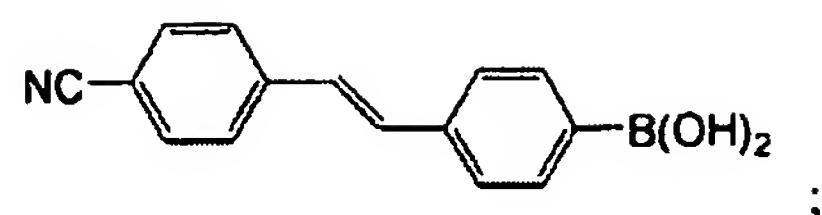


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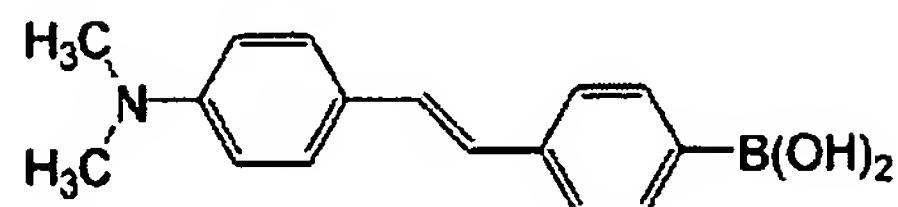


wherein X is chloride, bromide or iodide;

(VII)



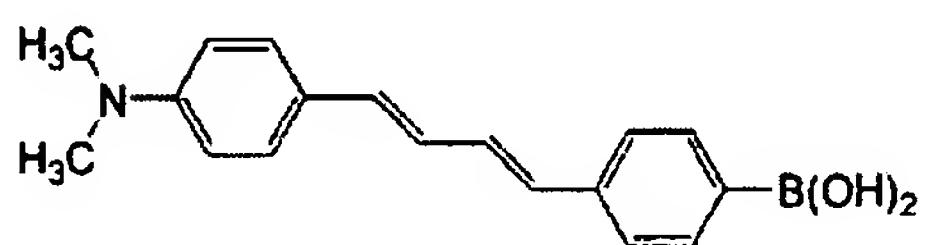
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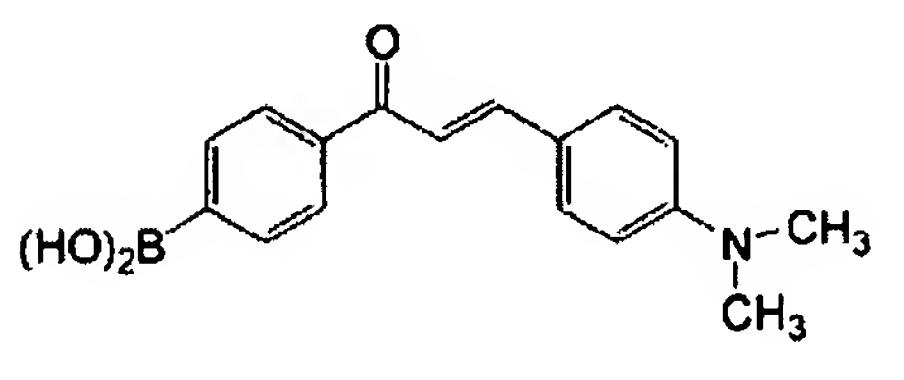
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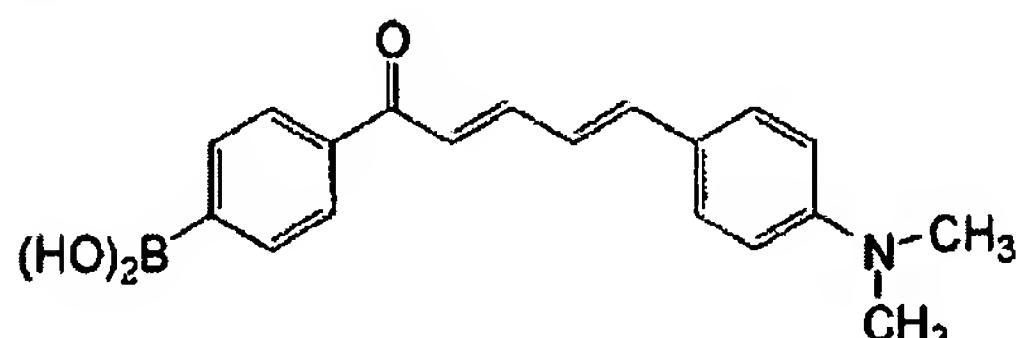


(X)



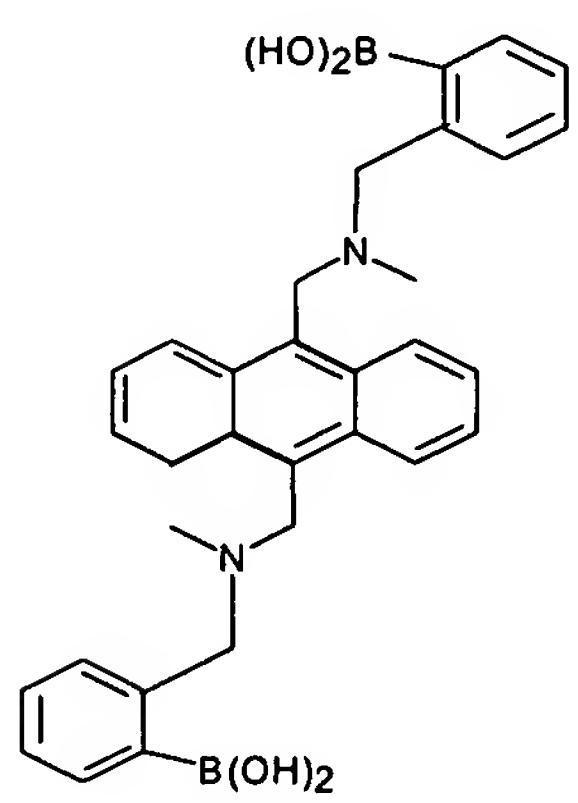
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(XII)



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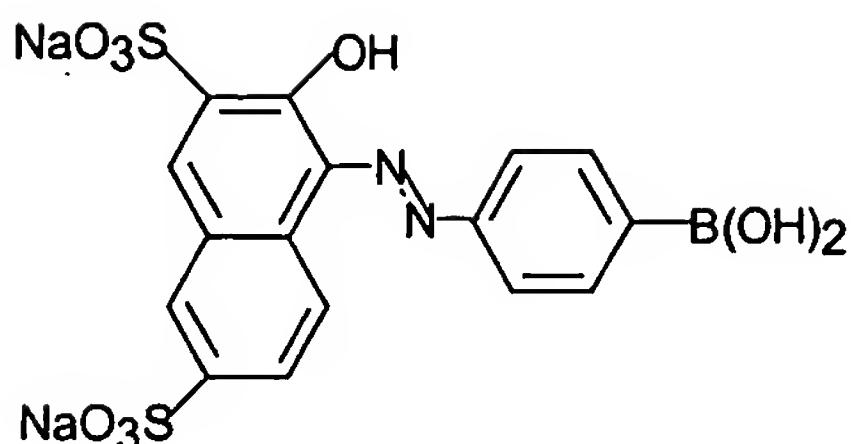
(XII)



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(XIII)

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14. The method of claim 1 wherein the change in the fluorescent property is visible in the
10 range of about 5 μ M to about 60 μ M cyanide concentration.

15. The method according to claim 7, wherein the cyanide concentration can be sensed at
levels less than 20 μ M.

15 16. The method according to claim 8, wherein the biological fluid is blood.

17. The method according to claim 1, wherein a colorimetric response is visible when
cyanide binds to the boronic acid containing fluorophore.

20 18. The method according to claim 1, wherein the step of observing made remotely by
incorporation of the boronic acid containing fluorophore as part of a fiber optic probe, wherein
the boronic acid containing fluorophore is attached to a fiber optic probe material.

19. A method for testing a biological or environmental test sample for the presence of a
25 cyanide compound, the method comprising:

- a) reacting a boronic acid containing fluorophore with the sample,
- b) illuminating the sample containing the fluorophore to generate an optical signal; and
- c) observing the sample with means for detecting the optical signal, wherein changes in the
30 optical signal indicates the presence of cyanide, wherein the boronic acid containing is
present in a concentration of about 100 nM to about 20 μ M; the cyanide is present in a
concentration of about 5 μ M to about 50 μ M; the illuminating step is accomplished using
excitation at a range from about 330 to about 370 nm; and the observing step is
accomplished using a fluorometer, fluorescence microscope, a laser scanner, or flow
cytometer.

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20. The method according to claim 19, wherein the optical signal is a wavelength shift that occurs when cyanide binds to the boronic acid containing fluorophore.

21. The method according to claim 19, wherein the illuminating step comprises use of a LED 5 comprising an excitation range from about 320 nm to about 400 nm.

22. The method according to claim 19, wherein the optical signal is a change in color, change in absorbance intensity, change of fluorescence intensity, a spectral shift, or a change in lifetime of fluorescence.

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23. A composition solution comprising a test sample suspected of containing a cyanide compound and at least one boronic acid containing fluorophore.

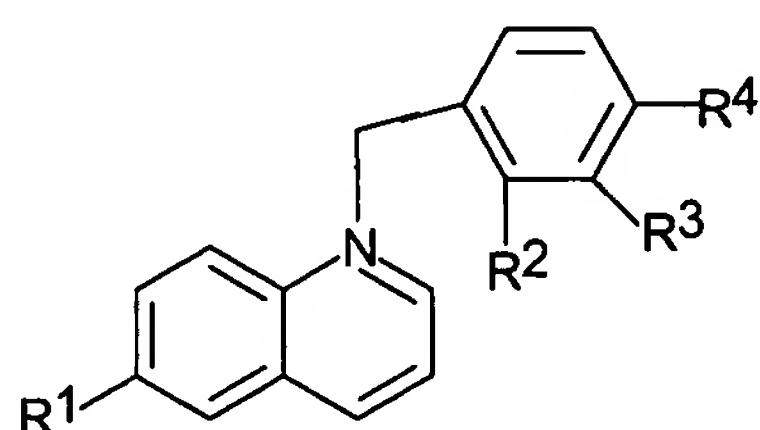
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24. The composition according to claim 23, wherein the boronic acid containing fluorophore comprises a heterocyclic quaternary nitrogen linked through a phenyl ring with a boronic acid moiety.

25. The composition according to claim 23, wherein the boronic acid containing fluorophore is

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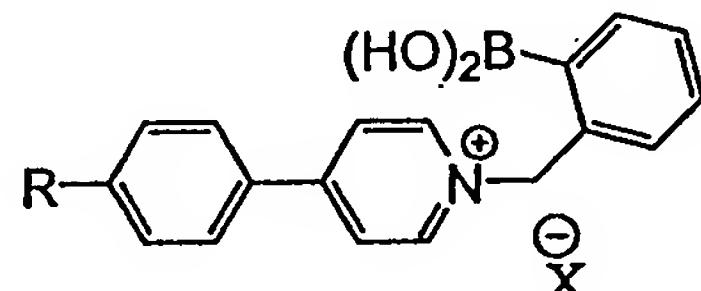
(I)



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wherein R¹ is H, a straight chain or branched C₁-C₄ alkyl group, C₁-C₄ alkoxy group, aryl group, or an amine group NR⁵R⁶, wherein R⁵ and R⁶ may be the same as or different from one another and is independently selected from the group consisting of H and C₁ – C₄ alkyl groups, and, R², R³ and R⁴ may be the same or different and may be hydrogen or B(OH)₂ with the proviso that the compound comprises one B(OH)₂ group;

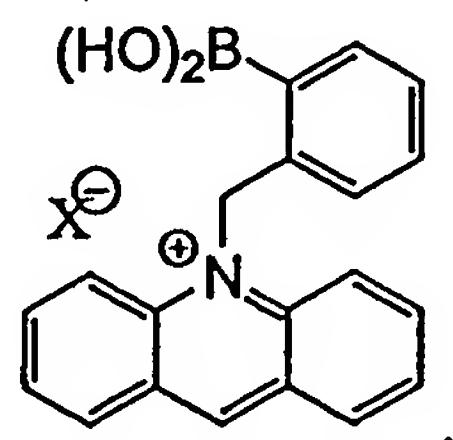
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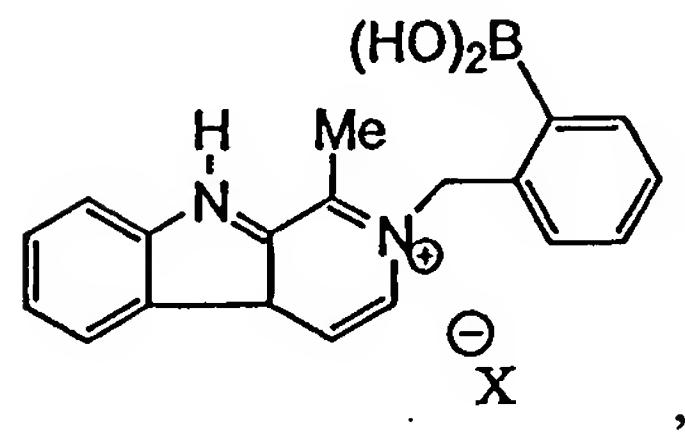
wherein X is chloride, bromide or iodide, and R is selected from the group consisting of H, straight chain or branched C₁-C₄ alkyl group, C₁-C₄ alkoxy group, aryl group, hydroxyl, sulfonyl, and NR⁵R⁶, wherein R⁵ and R⁶ may be the same as or different from one another and is independently selected from the group consisting of H and C₁ - C₄ alkyl groups;

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(III)

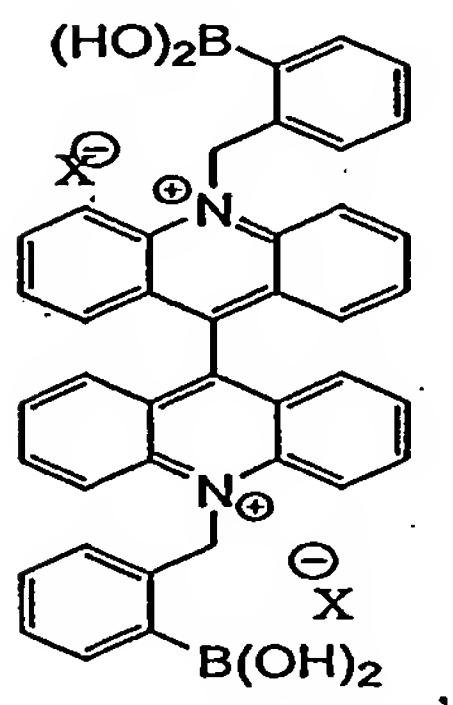


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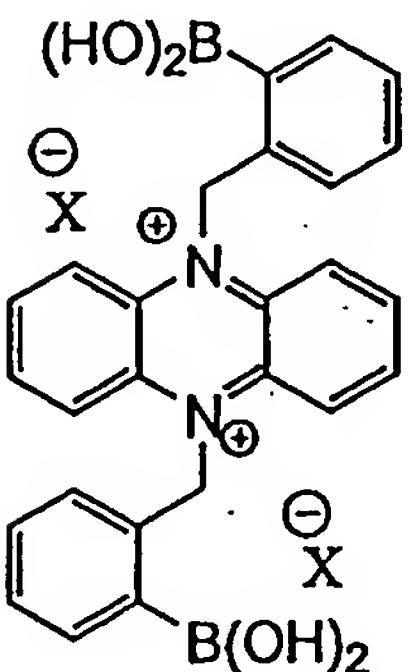


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(V)

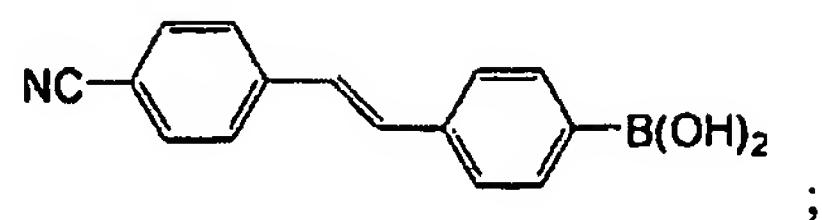


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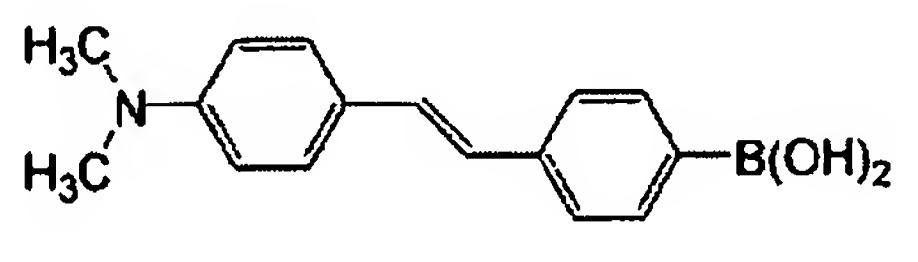


wherein X is chloride, bromide or iodide;

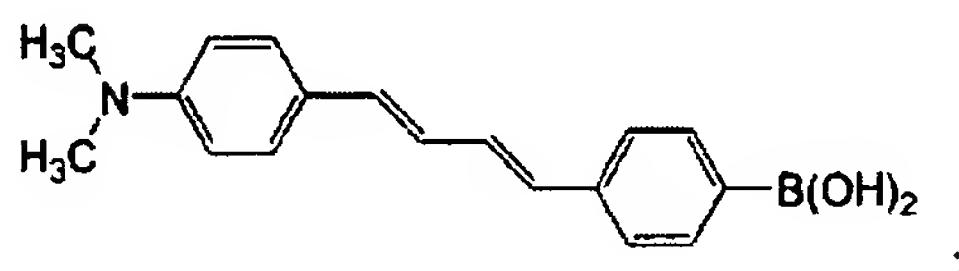
(VII)



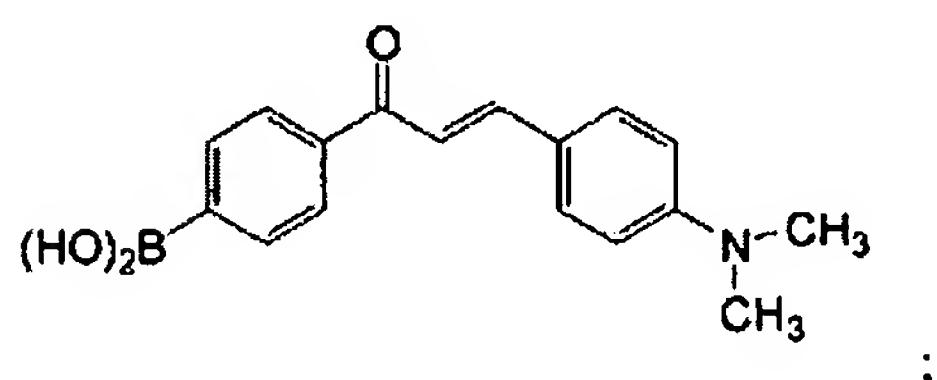
5 (VIII)



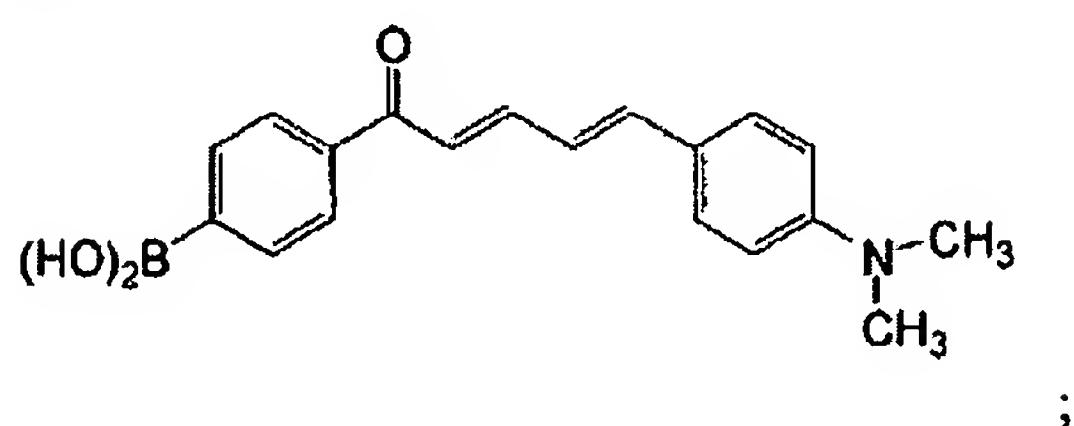
(IX)



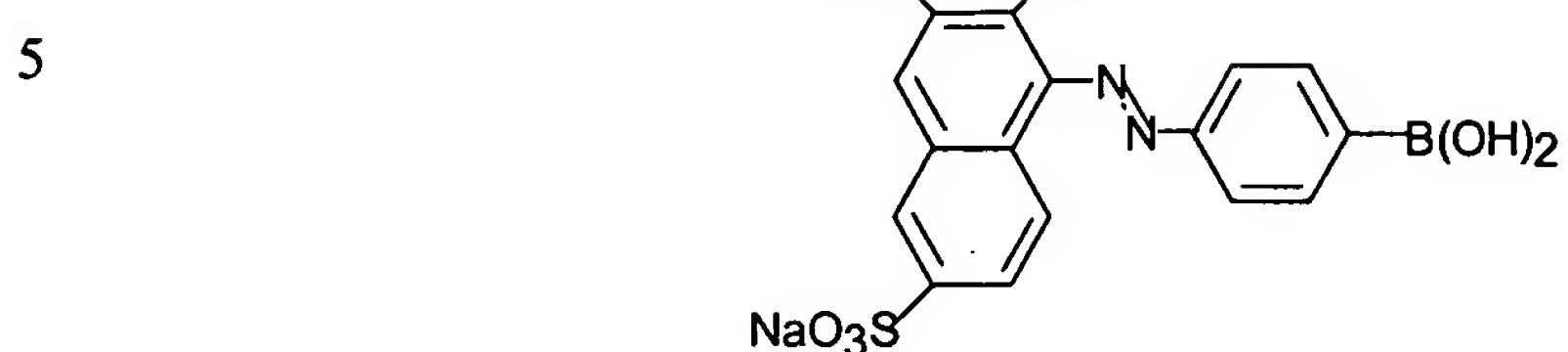
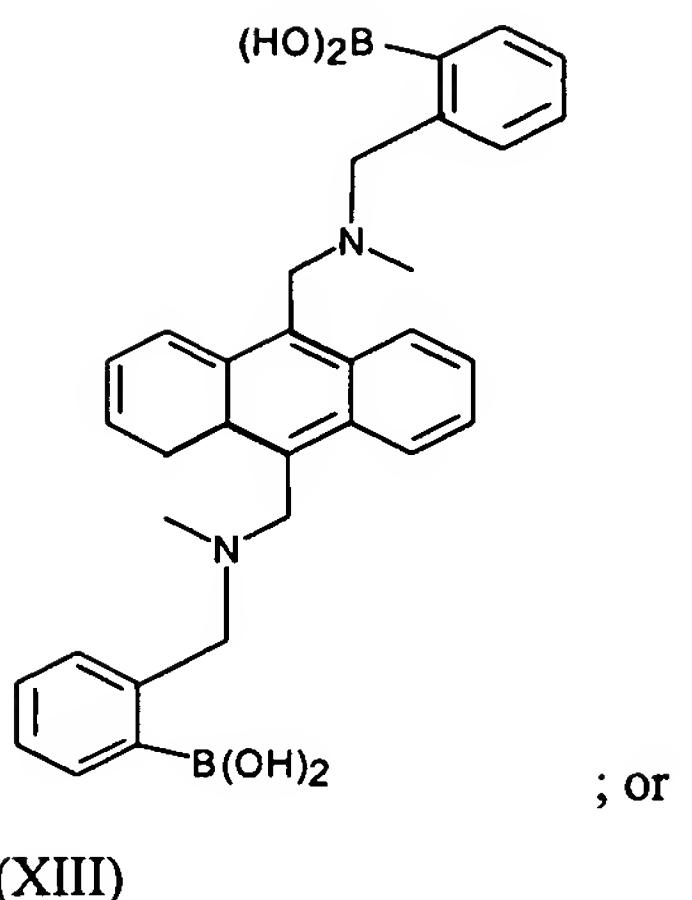
10 (X)



(XI)



(XII)



10 26. A method for testing a sample for the presence of a cyanide compound, the method comprising:
a) reacting at least one boronic acid containing fluorophore with the sample, wherein the boronic acid containing fluorophore is



20 and wherein R¹ is a straight chain or branched C₁-C₄ alkyl group, C₁-C₄ alkoxy group, aryl group, or an amine group NR⁵R⁶, wherein R⁵ and R⁶ may be the same as or different from one another and is independently selected from the group consisting of H and C₁-C₄ alkyl groups, and, R², R³ and R⁴ may be the same or different and may be hydrogen or B(OH)₂ with the proviso that the compound comprises one B(OH)₂ group;

25 b) illuminating the sample and boronic acid containing fluorophore to generate a response in a fluorescent property; and

c) observing the sample with means for detecting the fluorescent property, wherein a change in the fluorescent property relative to a control value for an unbounded free boronic acid containing fluorophore indicates the presence of cyanide.

27. The method according to claim 26, wherein the boronic acid containing fluorophore is *o*-BAQBA, -BAQBA, *p*-BAQBA, *o*-BMOQBA, *m*-BMOQBA, *p*-BMOQBA, *o*-BMQBA, *m*-BMQBA, or *p*-BMQBA.

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28. A kit for detecting and quantifying the amount of cyanide in a test sample, the method comprising:

10 at least one a boronic acid containing fluorophore in an amount sufficient to react with any cyanide in a test sample, wherein the boronic acid containing fluorophore is adhered to a solid support material, impregnated therein or in solution.

29. The kit according to claim 28, wherein the boronic acid containing fluorophore is attached to a fiber optic probe material or is attached to the fiber optic probe via an intermediate polymer.

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30. The kit according to claim 28, wherein the boronic acid containing fluorophore is *o*-BAQBA, -BAQBA, *p*-BAQBA, *o*-BMOQBA, *m*-BMOQBA, *p*-BMOQBA, *o*-BMQBA, *m*-BMQBA, or *p*-BMQBA.

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